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ABSTRACT

This autoinstructional lesson deals with the study of water pollution control. It is a learning activity directed toward high school students of biology and/or ecology. A general knowledge of microbiology techniques is regarded as a prerequisite for the lesson. Behavioral objectives are given. Emphasis is placed on use of techniques and materials to test for and identify specific bacteria. One hour is considered as the minimum time needed for the activity. The instructional packet includes a list of equipment needed, the type of space required, and a bibliography. A calculation chart, a cleanup procedure sheet, and a student evaluation form are included with the student script. (EB)

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TESTING WATER FOR BACTERIAL POLLUTION

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TEACHER'S GUIDE

PACKET NUMBER	591.5 D
SUBJECT	Biology, Ecology
TITLE	Testing Water for Bacterial Pollution
LEVEL	High School
PREREQUISITES	General knowledge of microbiology technique
BEHAVIORAL OBJECTIVES	1. To select stations along a stream for the collection of water samples for bacterial analysis. 2. To assess the impact of human activities on water quality. 3. To use the Millipore Environmental Microbiology Kit to test water for coligorm bacteria. 4. To count and identify coligorm bacteria. 5. To convert coliform counts to the number of coliforms/100 ml of water.
EQUIPMENT	Slide projector - 19 slides Cassette recorder - cassette tape Millipore Environmental Microbiology Kit Hot plate - alcohol Baby food jars Incubator (optional) Aerial photos and topographic maps Teacher's guide, script, 3 charts "Testing Water for Bacterial Pollution" by H. J. Dillner
TIME	1 hour +
SPACE REQUIRED	Carrel or laboratory counter
BIBLIOGRAPHY	American Public Health Assoc.,1971, Standard Methods for the Examination of Water and Waste Water, American Public Health Assoc.,Washington,D.C. Dillner, R.J.,1971 "Testing Water for Bacterial Pollution" unpublished teaching material prepared for the Univ. of Del. Population Curriculum Study.

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Millipore Corp., 1969, Experiments in Environmental Microbiology, Millipore Corp., Bedford, Mass.

SCRIPT

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Place SLIDE #1 in the viewer. (Pause) SLIDE #1 shows a view of the White Clay Creek which originates in Pennsylvania, flows past Newark and empties into the Delaware River south of Wilmington. The White Clay Creek, as well as other surface waters such as oceans, lakes, ponds, and rivers, contain various species of bacteria. In densely populated areas bacteria entering water from sewage is a serious problem. Diseases such as typhoid fever and dysentery can be traced to polluted drinking water. Since the organisms that actually cause diseases are difficult to detect, indicator organisms belonging to the easily detectable coliform group are routinely enumerated by health authorities as indicators of bacterial pollution. Coliform bacteria normally live in the intestines of animals, including man, but are not pathogenic. They are always present in sewage. Coliforms are very resistant organisms and are more difficult to kill than disease producers. Thus, if a sample of water is free of coliforms, one can be reasonably sure that it contains no pathogens. Drinking water should contain very few, if any coliforms and should be entirely free of pathogens.

Collecting stations for water samples should be carefully selected before going into the fields. This is best accomplished by map or aerial photograph study. Place SLIDE #2 in the viewer. (Pause) This slide shows a topographic map of the Newark area. Topographic maps show such features as buildings, roads, railroads, bodies of water, wooded areas and contour. Place SLIDE #3 in the viewer. (Pause) This slide shows

an aerial photograph of the same area as the topographic map. By referring to both the topographic map and the aerial photograph, it is possible to determine many factors which may affect water quality. For example: A stream which flows through a densely populated area is likely to contain sewage, and a stream flowing through an industrial area is likely to contain chemical wastes. Place SLIDES #4, #5, and #6 in the viewer. These slides show various human activities which affect water quality.

Place SLIDE #7 in the viewer. (Pause) This slide shows collecting stations on the White Clay Creek. Care in selecting collecting stations may yield important data. Locate stations 1 and 2 on the map. Data from Stations 1 and 2 may provide information on the bacterial input of the sewage treatment plant. Can you determine why collecting stations 3, 4, 5, and 6 are situated where they are? (Pause)

The next part of this lesson concerns the laboratory technique for analyzing water for coliform bacteria. Place SLIDE # 8 in the viewer. (Pause) This slide shows a Millipore Environmental Microbiology Kit being used in the field to test water for coliform. Although the kit is portable, you will be using it in the laboratory by bringing water samples to school. Water samples should be collected in small bottles such as baby food jars. Samples should be obtained from a part of the stream which is flowing. Do not take samples from along the bank where the water is muddy. Samples should be refrigerated and tested within 24 hours of collection.

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At this point in the lesson it would be wise to open the Millipore Kit and familiarize yourself with the equipment. A chart posted in the carrel shows the names of many of the components. Please turn off the tape recorder while you do this. (Stop)

The following procedure involves passing a measured volume of polluted water through a porous filter which traps all bacteria on its surface. The filter is then placed in a Petri dish with nutrients where colonies of bacteria become visible after incubation. The number of colonies present is then used to assess the degree of pollution. It must be stressed that asceptic technique must be followed to insure your personal safety since water samples may contain harmful bacteria.

Place SLIDE #9 in the viewer. (Pause) The first step in the procedure is to sterilize the filer holder and funnel by immersing them in boiling water for 3 minutes. Turn the tape recorder off while you perform this task. (Stop)

Place SLIDE #10 in the viewer. (Pause) The second step is to place a grided filter on the filter holder using forceps that have been sterilized by dipping in alcohol. Then screw the filter holder onto the funnel and place both on the receiver flask. Turn the tape recorder off while you do this. (Stop)

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Place SLIDE #11 in the viewer. (Pause) Insert a sterile absorbent pad in a Petri dish. These are located in the same envelope as the filters. Turn the projector off while you do this. (Stop) Place SLIDE #12 in the viewer. (Pause) Break open an ampoule of culture medium by placing the ampoule in the plastic breaker and squeezing it in your hand. Turn the tape recorder off while you do this. (Stop)

Place SLIDE #13 in the viewer. (Pause) Pour the entire contents of the ampoule onto the absorbent pad. Turn the tape recorder off now. (Stop)

Place SLIDE # 14 in the viewer. (Pause) Add about 20 ml of sterile water to the funnel. This water may be obtained by boiling tap water for 3 minutes. This water is added to the funnel to evenly disperse the bacteria in the sample which will be added to the funnel in the next step. Turn the tape recorder off while you add the water. (Stop)

Place SLIDE # 15 in the viewer. (Pause) Pipetta .5 ml of the stream water being tested into the funnel. Swirl the funnel gently with your hand to mix the bacteria. Turn the recorder off. (Stop)

Place SLIDE # 16 in the viewer. (Pause) Connect the two-way valve, syringe, and rubber tube to the receiver flask. This combination creates an effective vacuum pump. By pumping the syringe, a vacuum is

created in the receiver flask which causes water in the funnel to be drawn through the filter into the receiver flask. Any bacteria in the water is trapped on the surface of the filter. Turn the tape recorder off now. (Stop)

Place SLIDE #17 in the viewer. (Pause) Unscrew the funnel and by using sterile forceps, place the filter in the Petri dish on top of the absorbent pad. Replace the Petri dish cover. Turn the tape recorder off. (Stop)

Place SLIDE #18 in the viewer. (Pause) Invert the Petri dish and incubate it in the incubator at 37^{o} for 24 hours. The remainder of this lesson is to be completed after the 24 hours.

Place SLIDE #19 in the viewer. (Pause) Colonies should now be visible on your Petri dish. Only those colonies having a metallic sheen are coliforms. Turn the tape recorder off and count the coliform colonies. (Stop)

The number of coliform colonies you have counted represents the number of bacteria that were in the .5ml sample of water. Most health authorities express coliform counts as the number of bacteria per 100 ml of water sample. Let us assume that you counted 30 colonies on your Petri dish. To express your answer as the number per 100 ml follow the

procedure shown on the calculation chart which is posted in your carrel. Turn the tape recorder off while you perform this calculation. (Stop) The correct answer is 6,000 coliforms per 100 ml. Compute an answer using your own coliform count. Turn this data in to your instructor who will give you further directions. Be sure to follow the clean-up procedures which are posted. This lesson is now completed. Please turn off the tape recorder and rewind the tape.

CALCULATION CHART

$$\frac{\text{No. of Coliforms Counted} \times 100}{\text{Milliliters of Sample}} = \text{No. of Coliform/100ml}$$

CLEAN-UP PROCEDURES

Cultures, whether on filters or in any other medium, should be considered potentially dangerous and handled with utmost care

1. Remove the Petri dish covers using the back of the forceps as a prying tool. Put the covers and dishes (with cultures) into undiluted household bleach for 10 minutes.
2. Remove Petri dishes and covers using tongs or rubber gloves. Rinse under running water. Discard wet pads and filters.
3. Immerse the Petri dishes and covers in a solution of 70% alcohol for 10 minutes.
4. Remove the Petri dishes and covers. Allow them to dry on a paper towel. Reassemble the Petri dishes for reuse.

STUDENT EVALUATION FORM

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1. How should collecting stations be selected for bacterial stream analysis?

2. How do human activities affect water quality?

3. Briefly explain how to test water for coliforms using the Millipore Kit?

4. How do you identify coliform bacteria?

5. If you counted 23 coliforms in a Petri dish that were obtained from a 0.3 ml water sample, how many coliforms were there per 100 ml of sample?